ADRENAL RESPONSES IN A BLACK-TAILED JACK RABBIT (*Lepus californicus melanotis*) POPULATION

by

NEILS C. ANDERSON JR.

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INTRODUCTION AND REVIEW OF LITERATURE

Periodic population eruptions and the attendent economic importance of the Black-tailed Jack Rabbit (*Lepus californicus melanotis*) in Kansas have stimulated the interest and curiosity of field biologists for years. Consequently, several ecological studies have been made on black-tailed jack rabbit populations. This project was peculiar in that its scope included extensive study of many phases of a particular black-tailed jack rabbit population under essentially normal conditions. Studies were conducted on reproduction, pathological conditions, parasitism and physiological responses in terms of adrenal glands which provided the basis for this investigation.

The design of this study was to elucidate adrenal responses of black-tailed jack rabbits to such biotic and environmental characteristics and stressor as could be identified and accurately measured with the population. Study was made of histologically demonstrable adrenal lipoid and adrenal weights with respect to age groups, sex and a variety of extrinsic factors as temperature and precipitation.

Postulation of a functional disorder as the causative mechanism of periodic decimation of wild populations, has largely been responsible for the current emphasis on physiological ecology. Indirect study of physiological responses through adreno--cortical enlargement in terms of increased adrenal weight and discharge of cortical lipoids—which are well confirmed evidences of stress, greatly facilitates such investigations. Adrenal variations in terms of these criteria reflect the general physiological condition of the organism.

Physiological insights from adrenal research may ultimately prove of value in understanding the biological problem of mortality factors responsible for periodic population decline, but it is thought that any proposed

relationships would be premature in terms of our present state of knowledge of population fluctuations.

Attempts to explain the periodic population eruptions of black-tailed jack rabbits in Kansas have not been conclusive. According to Hall (1955), black-tailed jack rabbit populations in Kansas increase year after year and then die off in a short period of time with seemingly no regularity in population peaks. Marked oscillations in black-tailed jack rabbit populations have been observed in other areas too. Bailey (1931) in New Mexico, Taylor (1948) in Texas, and Kelson (1951) and Durrant (1952) in Utah have all given account of this interesting and economically significant phenomenon. According to Cahalane (1947) jack rabbit populations in most regions flactuate in cycles that average 7 years in length and range from 5 to 10 years. Wooster (1935), and Carter (1939) have shown a relationship between drought conditions in the 1930's and the increase in jack rabbits in western Kansas; and conversely, the jack rabbits were fewer during wet years. Anderson (1957) suggests that excessive precipitation inhibits growth of Danish hare populations. Bronson (1957) working with the jack rabbit population from which specimens for the investigation were collected (1958 a,b,c, 1959), has also postulated a direct correlation between increased numbers of black-tailed jack rabbits and drought conditions. He notes that the five year drought (1952-1957) was accompanied by a marked increase in jack rabbits in the area of this study. He further suggests, however, (1957) that such population increases may be more apparent than real for two reasons: first, animals tend to concentrate on cropland as a result of overgrazing and desiccation of their normal food supply; secondly, an actual decrease in the area of available habitat from overgrazing and an increased proportion of the

cropland in fallow further promotes concentration of the hare populations. Brown (1947) attributes the prevalence of black-tailed jack rabbits in western Kansas to their ability to adapt to changes in environment occurring as a result of extensive cultivation. Vorhies and Taylor (1933) have developed the concept of jack rabbits being an "animal weed", reasoning that increased numbers of jack rabbits is the result rather than the cause of rangeland depletion. Bond (1945) has come to much the same conclusion. He suggests that jack rabbits are more likely to be numerous on depleted and weedy range than on one in climax or near climax condition. Bronson (1947) concludes that there is no evidence to consider this population of jack rabbits cyclic. He suggests, however, that a long term investigation and study of the physiology of the animal may result in correlation of stage of succession of rangeland and population fluctuations.

Fluctuations in populations have probably been known from time immemorial. Historical records of such phenomena range from Old Testament accounts of locust plagues and rodent eruptions to the famed story of the Pied Piper. However it has only been in the last few decades that serious inquiry has been made into the mechanism of "cycle" causality (Hatfield 1940). The classical paper published in 1924 by Charles Elton (1924), attributing population cycles to sunspots, has been largely responsible for stimulating new interest and curiosity in population dynamics. The large literature which has been generated in an attempt to explain population "cycles" has been estimated at not less than 2000 publications (Cole 1956).

Investigators of cycles are divided into two schools of thought. One group demands no regular periodicity in their definition of cyclic behavior, and is inclined to consider population fluctuations as random occurrences

(Cole 1954). According to Palmgren (1949), fluctuations of population densities are largely explainable as a compound result of random variations of some master factors apparently climate and the influence of population density of the preceeding year.

The other school of thought, and traditional approach to the study of population fluctuations, describes cyclic behavior in terms of fairly regular fluctuations in numbers and recurrence with predictable frequency (Elton, 1942). Siivonen (1948), a Finnish worker, has summarized a large amount of data on fluctuations in numbers of a wide variety of organisms including several species of hares. He has concluded that the so-called 3-4-year cycle is the fundamental one for northern animals and the 10-year cycle is a summation of the shorter cycles.

Solomon (1949) has suggested that the cause for this divergence of opinions is two fold: first the tendency to embrace partial, one-sided explanations instead of seeking a comprehensive viewpoint, and secondly cleavage between the upholders of the primarily inductive approach, which according to Cole (1941), is the philosophical method field biologists are prone to use, and the deductive approach. Smith (1952) has concluded that we have a useful and valuable stockpile of concepts, but that at the present little of the experimental work is at all conclusive. Lack (1954) reminds us that even if cyclic fluctuations should be proved to be random the biological problem of mortality factors responsible for the decline remains.

In 1938, while carrying on extensive field and laboratory studies of the snowshoe hare (<u>Lepus americanus</u>), Green and Larson (1938a) observed

a syndrome of a functional disorder which they termed shock disease. It was determined that hares afflicted with shock disease had a disturbance of carbohydrate metabolism resulting from liver degeneration (Green and Larson 1938b). Green and Larson (1938a) suggest that shock disease was almost wholly responsible for the decimation of hares, however, according to Chitty (1959) there was insufficient evidence for such a conclusion.

This original thesis of a functional disorder as the causative mechanism in population crashes has been repeatedly confirmed by identification in wild and captive populations. Lechleitner (1958), who has recently conducted an extensive study of the black-tailed jack rabbit in California (1959a, 1959b) has observed the symptoms of shock disease in specimens which had been subjected to a variety of stressors such as wetness, cold, lack of suitable food, crowding, etc. He postulated (1958) that there may have been an exhaustion of the adreno-pituitary system resulting in hypoglycemic shock (shock disease) and death. Adams (1959) working with an isolated snowshoe hare population in Montana, observed a condition suggesting shock disease. However, since no pathologic diagonosis was performed, it was not certain the observed syndrome was the same. Christian and Ratcliffe (1952) observed the characteristic convulsive seizures and postmortum symptoms of the disease in several species of captive wild animals.

The tendency to consider shock disease as the universal single cause of periodic die-offs has not gone without critisim. Philip et.al. (1955) suggest that it is possible that a widespread disease following peak abundance is due to a combination of environmental pressures such as disease, parasitism and predation rather than to a single factor, shock disease.

The etiology of shock disease is still not fully understood. Develop-

ment of the general adaptation syndrome (GAS) by Selye in 1936 (Selye 1936) has proved to be the most important single factor in interpreting the disease syndrome. The basic premise of this adaptation reaction is that an organism responds with a sterotyped reaction pattern during stress which is independent of the nature of the damaging agent and hence represents a response to stress as such (Selye 1937). The GAS comprises three distinct stages: the alarm reaction which is characterized by necrosis of the liver, marked hyperplasia of the adrenal cortex along with a rapid loss of cortical lipoid, and sudden involution of the thymus; the stage of adaptation or resistence characterized by a reversal of the syndrome precipitated by the alarm reaction and the exhaustion phase which occurs when the animal succumbs to the sustained stress and is characterized by the symptoms of the alarm reaction (Selye 1937). It should be born in mind that the GAS is essentially a physiologically useful phenomenon, However, Selye (1951) has proposed that derangements of this mechanism result in certain abnormalities which he considers to be essentially diseases of adaptation.

In the light of Selye's work and a comprehensive picture of the shock disease snydrome, Christian (1950) has suggested that shock disease as described by Green is the manifestation of the exhaustion phase of the adaptation reaction. Christian (1950) has combined these concepts and has postulated a physiological mechanism controling mammalian population growth and decline. This thesis considers socio-psychological pressures as stressing stimuli and that they occur in some proportion to population density (Christian 1957). The concept of population density developed by Christian (1957), suggests that measurable physiological responses occur in individuals with increasing population size and that this is the result of

increased social competition. According to Chitty (1952, 1954) crowding increases social competition and causes a state of psychological excitement which is transformed by the adreno-pituitary system into physical stress. Selye (1937), Christian (1955a, 1955b) have shown that stress activates the adreno-pituitary system and suppresses reproduction. Selye (1939) postulates that adrenocorticotropic hormone (ACTH) production is at the expense of gonadotrophins under stressed conditions hence accounting for the reproduction failure. It has also been shown that crowding and its attendent stress adversely affects offspring. Chitty (1954) first postulated this to be an intra-uterine effect with permanent damage to surviving young. Christain and Lemunyan (1958) have shown that suppression of lactation is the principle cause of stunting and other effects on offspring. According to Retzlaff (1938) dominant females give better reproductive performance suggesting the importance of behaviral factors in reproduction.

The effect of environmental factors limiting population growth, according to Christian (1957), are superimposed on and operative through this socio-psychological-physiological system. He further postulated that when in free wild animal populations the combined demand of social competition and environmental factors exceed the maximum native capacity of the organism to adapt, the endocrine adaptation mechanism breaks down resulting in death. Christian (1952) has termed this adreno-pituitary exhaustion which he considers to be a disease of adaptation. The triggering mechanism according to Christian (1950) involves activation of the complex hypothalmo-hypophyseal-adrenocortical-gonadal system (Selye 1954, Harris 1956). Frank (1957) in working with microtene cycles in Germany, postulates that additional meterological stressors such as frost periods, result in disturbing the adreno-pituitary system.

It has been well established that adrenal enlargement is evidence of increased adrenal activity, and that this increase is the result of an increase in cortical tissue (Selye 1936, 1937). Consequently the amount of cortical tissue, as measured by adrenal weight, is an indirect measure of stress (Christian and Davis 1955). These authors also suggest that for a particular population adrenal weight itself might be used as an index of relative density provided that adrenal weights form a series of known population densities are available with which to compare those from the unknown population. Using populations of Norway rats (Rattus norvegicus), they were able to demonstrate this relationship by reducing population size and measuring the concomitant reduction in adrenal weight.

Tarantine and Cassano (1955) working with rats have shown that pregnancy results in an increase in adrenal weight. Donaldson (1924), on the other hand, claims that there is no adrenal enlargement during pregnancy and lactation in the rat. Donaldson (1919) working with rats observed a rapid decrease in the relative volume occupied by the medullary tissue after birth. Masui and Tamura (1924) demonstrated that the size of adrenal glands of female mice differ considerably according to various periods of the estrous cycle, and this variation in size was due to the structural difference in the gland. According to Jackson (1913) there is a sexual difference in adrenal size in albino rats with the adrenal bodies of the female being larger.

Discharge of histologically demonstrable lipoids in response to increased adrenal activity during stress has also been well demonstrated (Zwemer 1936, Selye 1936, and Dalton, Dosne and Selye 1940). There is considerable confusion in the literature as to the terminology applied to the study of fatty

substances (Lee 1937). According to Whitehead and Kay (Lee 1937) each of the words fat, lipoid, lipid, lipin, may denote either any fatty substance in general or one or more groups of fatty substances defined according to chemical properties. They have proposed the use of the term lipoid in reference to histological examinations to denote sudanophilic substance. Selye (1936) has shown that the alarm reaction is characterized by a sudden decrease in cortical lipoids, but that the continuation of the alarming stimulus results in the reappearance of the sudan staining granules, which are more evenly distributed throughout the various layers of the cortex. At the end of the alarm stage, according to Selye (1936), lipoids concentrate in the zona fasciculata. He has also shown (1937) that during the stage of resistance the adrenal cortex remains loaded with lipoids. The initial loss of lipoid is apparent upon gross inspection since the organ loses its characteristic light yellow color and turns dark brown, in fact, this large brown adrenal is the most typical macroscopic sign of the alarm reaction (Selye 1936). Fortier et.al. (1950) conducted extensive studies on the chemical and morphological changes elicited in adrenals of rats by stress. In rats subjected to severe systemic stress over a 48 hour period there was an almost complete depletion of cortical sudanophilic material. Rats which had been fasted and subjected to severe cold for a 24 hour period showed an intense discharge of sudanophilic substance from the adrenal cortex. This group of rats also showed the highest degree of adrenal enlargement. According to Christian (1950) the quantity of lipoids contained in the cells of the zona fasciculata of animals that died of shock disease usually decreased with the duration of stress. Howard Miller (1927) has conducted extensive study of the transitory zone of the adrenal

cortex of the mouse paying particular attention to lipoid concentrations and distribution according to age and sex. Whitehead (1934) has made extensive and systematic study of the variations in the cortical lipoids of guinea pig adrenals with age and sex. He has shown that female guinea pigs have adrenals containing more stainable lipoid than the male. He has also coined the term "lipoid-laden area" which denotes the band of the cortex demonstrating sudanophilic properties.

The reliability of histological techniques for the determination of total or differential lipoid content of tissues as compared with chemical methods has been questioned repreatedly (Knouff et.al. 1941). However, Kay and Whitehead (1935) working with rabbits and Mayer et.al. (1912) working with the horse, found no discrepancy between the histological and chemical findings. Another criticism of histological techniques is their failure to stain all fatty material present. Hoerr (1936) has suggested that this "masked lipoid" fraction is a constant constituent of most tissues and has the same function in the adrenal as in other cells. He also postulates that it is the histologically demonstrable lipoids that are concerned primarily with the specific functions of the adrenal cortex.

MATERIALS AND METHODS

Procedures employed in this phase of the ecological study included collection of specimens, dissection of the animals in the field laboratory with removal and fixation of the adrenal glands, and interpretation and further study of the raw field data and materials. Laboratory work included weighing, sectioning and histological examination of the adrenal glands.

Random samplings of the jack rabbit population within a 10 mile radius

north of Lakin, Kansas, in Kearney county, provided most of the specimens for this study. Collections were made at night when the animals were normally active with the aid of a 12 volt spotlight. Animals were shot using a 22 caliber rifle or occasionally, a 12 gauge shotgun.

Hunting success appeared to depend largely on activity within the jack rabbit population. Periods of severe change in temperature, fog, and heavy precipitation were factors in limiting activity. However, within the limits of seasonally normal conditions, population movements were independent of meterological factors. An exception to this was wind velocity, which, even in moderation, sharply curtailed activity of the animals.

Also, due to the nature of the collecting technique, the random sample may show an age ratio bias. Bronson (1957) has estimated that jack rabbits are at least a month old before they begin to appear in the collections.

The adrenal glands of the black-tailed jack rabbit are easily located in the eviscerated animal. They are suprarenal in location and usually imbedded within some fatty tissue. A little experience enables one to dissect them out without difficulty and without rupturing the extensive vascularity in the area. Under the handicap of field conditions, it was not feasible to weigh the adrenal glands as fresh tissue consequently they were fixed in 10 per cent neutral formalin and brought back to the research laboratory for trimming and weighing. This technique has the advantage of eliminating the possibility of unequal drying during trimming and handling. The trimmed, formalin-fixed adrenals were drained on filter paper in a standard manner and weighed in an inclosed micro-balance to 0.1 mg.

These weights represent only the amount of insoluble tissue constituents rather than the true value for the living gland, but have the

advantages that varying amounts of intercellular and intracellular aqueous fluids are not included and that the figures obtained are of significant accuracy and are suitable for comparison with each other.

Histological study of tissue lipoid material demands a sectioning and staining technique which does not involve use of organic solvents commonly employed with paraffin and celloidin sections. The freezing microtome has been employed almost exclusively for such studies. The use of this technique, along with the proper fixative, entirely eliminates tissue contact with fat solvents hence keeping loss of lipoid materials at a minimum. The freezing microtome, however, does present certain disadvantages for histological study. The structural elements of the tissue may be disorganized; it is practically impossible to obtain and correlate serial sections and really thin sections are difficult to obtain. This latter factor presents a particular handicap in many studies since sections of 10-15 u do not facilitate study of histological detail. However, since this study was not directed at detailed study of the cellular morphology of the adrenal cortex, the thickness of the sections was of little consequence as long as they were consistently of uniform thickness.

A systematic study of the variation in cortical lipoid in terms of distribution and density was performed with respect to age and sex. The terms "lipoid-laden cortex" and "lipoid-laden area" are used to denote the darkly stained band in the cortex visible in plate III, Fig. 1. The subcapsular rim of cells and cortical cells scattered throughout the medulla were not regarded as part of the "lipoid-laden cortex" because their total lipoid content was very small.

The subjective nature of this quantitative study of cortical lipoids,

demands unusual consideration of detail to keep the inherrent human factor at a minimum and reproducibility maximal. It is believed that discussion of laboratory techniques in detail is not only appropriate but necessary to support the histological data presented.

Freezing Solution

This reagent, essentially a sugar solution, is analogus to the embedding media of praffin sections. It functions in supporting the tissue during sectioning and also provides a base for attachment of the tissue to the stage during freezing. "Hamilton's freezing mixture" (Clayden 1948) was used and is prepared as follows:

Solution A.
pure cane sugar ———————— 142.5gm.
dist. water ———————— 150.0ml.

Solution B.
gum acacia ———————— 2.8gm.
dist. water ———————— 150.0ml.

For use, equal parts of A and B are mixed. The separate reagents are stored in the refrigerator.

Gelatin Coated Slides

To facilitate manipulation of the sections during the critically timed staining procedure, they were affixed to gelatin coated slides. The slides were prepared by placing two drops of 2 per cent gelatin on one end and spreading it much as one prepares a thin blood smear (Lee 1937). They were immediately transfered to an upright slide box and allowed to dry. Accumulation of stain on the coated slide indicates an excess of gelatin.

Sudan III Stain Solution

There are a variety of solvents available for preparing oil-soluble stains as sudan; however, they are usually made up in 50-70 per cent alcohol (Gomori 1953). It was found in this laboratory that a relatively low concentration of alcohol, 50 per cent, with acetone, 1 part/10, produced optimal staining in 5 minutes and resulted in sharp differentiation of lipoid distribution and density within the lipoid-laden area of the adrenal cortex. The stain used was prepared as follows: a satured solution of Sudan III in 50 per cent alcohol was prepared by heating--approximately a 2 per cent solution. This was colled and filtered. Just prior to use acetone was added in the proper proportion. Since the stain was unstable, a new solution was prepared if more than a day elapsed between stainings. The differential in volatility between the two solvents also necessitated frequent change of stain solution to maintain a constant fat solvent strength thus insuring equal staining from one series of slides to the next.

Mounting Medium

Zwemmer's "Glycrogel" (Lee 1937) The usual mounting media contain organic solvents and consequently are not applicable to sudan stained sections. Zwemer's "Glychrogel", a gelatin base solution, is designed primarily for frozen sections and was prepared as follows (Lee 1937):

glycerin	20 ml.
gelatin, granulated	3 gm.
chrome alum	0.2 gm.
dist. HOH	80 ml.

This preparation is refrigerated and for use is liquified by placing the

container in hot tap water.

Sectioning and Staining Techniques

Thin, median portions of the formalin fixed adrenals were cut with a razor blade. Before sectioning these tissue slices were "soaked" in the freezing solution. They were then transferred to the freezing stage of the microtome along with a drop or two of the freezing mixture.

Freezing was accomplished by short bursts of CO_2. The blade was now brought into juxtaposition to the frozen tissue and set to cut at 15u. The first few sections were discarded to assure uniform thickness. Uniform sections were removed from the blade and placed in a beaker of distilled water. The next few sections were again discarded to avoid a thickness differential resulting from expansion of the tissue upon thawing.

The sections were floated onto the gelatin coated slides, the excess water drained off, numbered, and set aside to dry. They were then ready for staining. Two slides were made from each adrenal sectioned.

The staining procedure employed was a modification of that outlined by Clayden (1948) for demonstrating lipoid materials in frozen sections.

1) Affix tissue sections to gelatin coated slides
2) Wash slides in distilled HOH for 3 minutes
3) Rinse in 70 per cent alcohol 10 seconds
4) Stain in Sudan III for exactly 5 minutes
5) Rinse in distilled HOH for 2 minutes
6) Allow slides to become nearly dry at room temperature
7) Mount

Three divisions of the lipoid-laden area of the cortex were observed to be readily and consistently demonstrable in terms of staining intensity. No attempt was made to identify these zones histologically with the usual morphological zoning of the cortex. The proportion of the cortex occupied

by a particular zone varied greatly, however, suggesting a correlation with the various proportions of cell types comprising the cortex under differing states of activity. Both adrenals from a series of 11 rabbits were sectioned to determine the homogeneity of the two in terms of fat density and distribution. The results showed that if there were a fat differential between the left and right adrenals, it could not be determined histologically. Left and right adrenals were not distinguished, therefore, in choosing a gland for sectioning.

In order to arrive at an estimation of lipoid density and distribution within the cortex, it was necessary to set up criteria which would reflect quantitative measurements. To establish the zones of lipoid distribution it was decided to assign the letters A, B, and C to the outer, median, and inner lipoid-laden zones of the cortex respectively. Lipid density within the individual zones was estimated by assigning to each a numerical value of 0-3 according to the intensity of the staining as observed microscopically.

Due to the subjective nature of these estimations, it was desirable to design a check on the consistency of the readings. To this end each series of slides was graded separately from its complement and the two readings compared. When there was a discrepancy both slides were observed simultaneously and the difference resolved.

In general, statistical analysis consisted of a regression correlation between adrenal weight and various biotic and environmental factors in an attempt to account for variation in adrenal weight in terms of these measurable intrinsic and extrinsic stress factors.

Because the monthly collections were not made exactly at 4 week intervals or consistently at the beginning or end of the month, it was decided

that monthly weather data tabulations would not be valid for correlation study. Meterological data for a 30-day period prior to each collection were calculated and used in such statistical analysis.

EXPERIMENTAL RESULTS

The adrenals of the black-tailed jack rabbit occupy a suprarenal position and are usually imbedded in some fatty tissue. Macroscopically, freshly exposed adrenals usually demonstrate a characteristic light yellow color. There was considerable color variation among specimens, however, ranging from white to medium brown, (Plate I, Lower). Occassionally, hemorrhagic areas were observed on the surface of the gland. Variation in size between the right and left adrenals was readily recognized during dissection.

Fresh adrenal tissue was observed to undergo degredation rapidly when exposed to normal room conditions. Characteristically such tissue developed a dark brown coloration. Formalin fixation arrested color change suggesting maintenance of lipoid integrity within the cortical tissue.

Microscopic examination of the gross morphology of adrenal sections showed that the total area of medullary tissue did not vary greatly among specimens, while the relative proportion occupied by cortical tissue varied over a wide range (Plate II).

Anomalies of cortical structure were not infrequent (Plate III). Occasionally, cortical invaginations extended nearly to the medulla. Macroscopic examination of certain intact glands also showed areas in which there was literally no cortical tissue as evidenced by invagination and the absence of the characteristic cortical color.

Cortical lipoid was defined as that substance in the cortex stained

by Sudan III. Microscopic study showed that lipoid density, in terms of staining intensity, was proportional to the concentration of lipoid materials within the cellular elements. Those sections demonstrating emulsification of lipoid substance characteristically stained lighter.

The adrenal cortex, all or part of which may be sudanophilic, (lipoid-laden area), was shown to be divisable into three zones according to staining intensity. Immediately adjoining a subcapsular rim of cells was the outer cortical division, zone A. Lipoid in this area was usually at minimal density (Plate III, Fig. 1.). Greater and more uniform lipoid concentration in zones B and C resulted in a decreased stain differential which in certain instances obliterated the border between the two zones. The proportion of the cortex occupied by these divisions varied, however, the relative breath of the individual zones for a particular adrenal was constant.

Cortical cells were seen mingled with those of the medulla in most sections (Plate II, Fig. 1). The sudanophilic properties of this tissue corresponded with that of the inner lipoid-laden zone. Medullary tissue did not stain with Sudan III.

Distribution of Age and Sex Groups and Corresponding Mean Adrenal Weights with Lipoid Density Within Zones (Table 1)

Lipoid density within the cortical zones showed definite variations with age and sex.

The distribution of lipoid density in zone A of adrenals removed from pregnant adult females showed the estimation of "1" to be the most frequent reading. This group also had the highest percentage of individuals exhibiting no sudanophilic material in this area of the cortex. The mode lipoid

density of non-pregnant adult females for zone A was also "1", but none was found to be devoid of sudanophilic substances in this area. Also, the frequency of zone A maximal lipoid density was the highest of all groups studied in non-pregnant adult females. The zone A mode for adult males was "2", however, this group also contained a relatively high frequency of individuals demonstrating no staining in this area. The majority of juveniles demonstrated zone A lipoid density of "1" and correspondingly had a relatively high frequency of individuals devoid of staining in this area and a low percentage with maximal staining.

There was no individual that failed to demonstrate sudanophilic properties in zone B of the adrenal cortex. The most frequent reading for all groups was "2". Again, as in zone A, the pregnant adult females tended to have less lipoid than non-pregnant adult females. Adult males showed a relatively close correlation with zone A in terms of the frequency of individuals with "1" and "2" lipoid density.

The frequency of maximal lipoid density increased in all groups except pregnant adult females in zone C. No individual failed to demonstrate some sudanophilic materials; and non-pregnant adults did not show lipoid density below the estimated value of "2", while 45.9 per cent demonstrated maximal staining. Compared with pregnant adult females the trend for less lipoid in pregnant animals again was noted. Adult males showed a marked increase in the frequency of maximal lipoid density while juveniles showed little variation from the zone B readings demonstrating relatively small increment.

A simple regression statistical analysis between adrenal weights of age and sex groups and zone lipoid-density-index-numbers showed the two to be independent of one another.

Table 1. Distribution of age and sex groups and corresponding mean adrenal weights with lipoid density within zones.

Lipoid-Laden Zone		A				B				C			
Lipoid Density Index Number		0	1	2	3	0	1	2	3	0	1	2	3
Pregnant Adult ♀	%	17.2	39.4	33.7	9.5		23.8	54.8	23.8		4.8	71.4	23.8
	Average Adrenal Weight	345.1	407.9	379.0	404.6		374.1	385.8	447.2		253.1	384.3	447.4
Non-Pregnant Adult ♀	%		44.0	42.0	14.0		8.3	62.5	29.1			54.1	45.9
	Average Adrenal Weight		379.0	392.6	346.3		353.3	363.3	419.0			388.1	387.4
Adult ♂	%	10.5	32.9	50.0	6.6		35.5	47.4	17.1		7.9	51.3	40.8
	Average Adrenal Weight	282.6	262.6	276.3	234.4		267.8	277.1	252.6		297.7	262.3	273.7
Juveniles ♂ and ♀	%	13.0	33.9	51.0	2.1		9.8	67.1	23.1		6.3	64.8	28.9
	Average Adrenal Weight	242.9	227.1	244.2	311.8		202.9	237.2	247.2		204.6	236.3	248.8

Variation in Total Lipoid with Age and Sex (Table 2)

Summation of the zone lipoid-index-numbers provided the basis for estimating total lipoid content of the adrenals. Pregnant adult females were further classified according to the stage of pregnancy, adult males according to the state of sexual activity and juveniles were grouped according to sex.

It was found that pregnant adult females at less than half term had a minimum total-lipoid-index of "4", and 40 per cent registered a value of "6". Those at half term or over demonstrated total-lipoid-index-values from "1" to "9" the mode being "6". Certain of those individuals at half term or over demonstrated very low total lipoid content whereas those in earlier stages of pregnancy failed to show such lipoid depletion and at the same time the frequency of individuals with maximal total lipoid was greater.

The relationship between scrotal and non-scrotal adult males in terms of total lipoid was variable. The mode of scrotal individuals was "6" and that of non-scrotal animals "5". Adult females showed a slightly greater total lipoid content then adult males; juvenile females had a mode total-lipoid-index number of "6" and juvenile males "5", thus demonstrating the same relationship.

Table 2. Variation in total cortical lipoid with age and sex.

Total Lipoid	Adult Females				Adult Males			Juveniles		
	*Pregnant		Non-			Non-				
	$<\frac{1}{2}$ term	$>\frac{1}{2}$ term	Pregnant	Total	Scrotal	Scrotal	Total	Male	Female	Total
1		2.7		1.0	1.1		0.9			
2		2.7		2.1	1.3		1.1	2.4		1.2
3		13.5	3.0	6.3	4.4	5.5	4.5	4.9	6.0	5.4
4	8.0	10.8	3.0	7.3	10.0	16.7	10.5	12.2	10.7	11.4
5	24.0	21.6	20.6	21.9	26.2	38.8	28.6	32.9	31.0	31.9
6	40.0	24.3	38.2	33.3	35.4	5.7	30.5	25.6	32.1	28.5
7	12.0	16.2	17.6	15.6	13.6	22.2	15.4	17.1	14.3	16.2
8	10.0	5.4	11.8	8.3	6.8	11.1	7.5	3.7	6.0	4.8
9	6.0	2.8	5.8	4.2	1.2		1.0	1.2		0.6
Mode	6	6	6	6	6	5	5.5	5	6	5.5

*According to Lechleitner (1959) black-tailed jackrabbit embryos of 40 mm crown rump length are one-half term. This provided the basis for the classification of less than one-half term ($<\frac{1}{2}$ term) and over one-half term ($>\frac{1}{2}$ term).

Frequency of Uniform Staining Throughout the Cortex and Absence of Lipoid from Zone A within Age and Sex Groups (Table 5)

Analysis was made of the frequency of uniform staining in the cortical zones and failure to demonstrate sudanophilic materials in the outer area of the cortex. Results showed that 40 per cent of the adult pregnant females at less than half term demonstrated uniform staining throughout the cortex, and 8 per cent were devoid of lipoid in zone A. Pregnant individuals at half term or over showed 24.2 per cent with uniform staining and an equal proportion lacking sudanophilic materials in zone A. The frequency of non-pregnant adult females demonstrating equal staining was relatively high (19.5%) and in no instance did they fail to have lipoid in zone A.

Scrotal males showed a significantly higher frequency of uniform staining than non-scrotal adult males, whereas the reverse relationship was true for the absence of staining in zone A. Juvenile males and females did not show significant differences in either measurement, although they did demonstrate a significant frequency of uniform lipoid distribution and absence of lipoid from the outer area of the cortex as a group.

Table 3. Frequency of uniform staining throughout the lipoid-laden area of the cortex.

Adult Females			Adult Males		Juveniles	
Pregnant		Non-		Non-		
$< \frac{1}{2}$ term	$> \frac{1}{2}$ term	Pregnant	Scrotal	Scrotal	Male	Female
40.0%	24.2%	26.4%	19.5%	5.5%	20.0%	22.6%
31.0%			17.1%		20.7%	
	29.3%					

Frequency of absence of staining from zone A.

8.0%	24.2%	0.0%	9.2%	16.7%	11.8%	13.1%
17.2%			10.5%		13.0%	
	10.9%					

Adrenal Weight Variations with Age and Sex and in Response to Biotic and Environmental Factors

Adrenal weights of black-tailed jack rabbits showed definite variations with age and sex. Adult pregnant females demonstrated a greater mean value than non-pregnant adult females, adult females greater than adult males, juvenile females greater than juvenile males and adults greater than juveniles (Table 4). In all groups the left adrenal weighed consistently more than the right.

Variation in Adrenal Weight with Age and Sex

Adult Female Pregnant	Adult Female Non-Pregnant	Adult Male	Juvenile Female	Juvenile Male
388.4	378.8		245.9	229.3
		270.0		
383.6			236.2	

Figures are mean total weights of left and right adrenals in milligrams.

Multiple regression techniques applied to female jack rabbit data accounted for 52.4 per cent (Table 5) of the variation in adrenal weight. Reproductive factor correlations with adrenal weight were all of significant magnitude. Occurence of a previous pregnancy (parous) ranked highest followed by pregnancy, age, and body weight. Number and length of embryos correlated significantly with adrenal weight but ranked relatively low in respect to influence on adrenal weight variations.

Meterological data in terms of average temperature, frost days (32°F or below), and days 90°F or above correlated significantly with male adrenal weights, whereas precipitation did not demonstrate a significant correlation (Table 6). Age and body weight correlations with adrenal weight ranked high paralleling the female correlations. Activation of the testes (scrotal)

showed significant positive correlation with adrenal weight. Total variation in male adrenal weight accounted for by multiple regression techniques was 16.6 per cent.

Table 5. Simple correlation of biotic factors with female black-tailed jack rabbits.

	*	1	2	3	4	5	6	7	8	Rank of Factors Influencing Adrenal Weight
Age (Juv. - Ad.)	1	1	.663	.985	.705	.594	.529	.408	.644	2
Pregnancy	2		1	.675	.550	.895	.798	.260	.486	4
Parous	3			1	.703	.604	.539	.416	.658	1
Body Weight	4				1	.546	.514	.405	.627	3
Number of Embryos	5					1	.772	.264	.474	5
Length of Embryos	6						1	.206	.434	7
Lactation	7							1	.472	6
Adrenal Weight	8								1	

N - 120, d f. - 118 Critical Valve at 5% Level - .180

52.4% of the variation in adrenal weights was accounted for by using all 7 variables and multiple regression techniques.

*Column identification is the same as for rows.

Table 6. Simple correlation of biotic and environmental factors with male jack rabbits.

	*	1	2	3	4	5	6	7	8	Rank of Factors Influencing Adrenal Weight
Age (Juv. - Ad.)	1	1	.476	-.540	-.442	.507	.162	.850	.357	1
Body Weight	2		1	-.417	-.447	.274	.265	.460	.273	5
Average Temperature	3			1	.849	-.894	.203	-.442	-.335	2
Days 90° or above	4				1	-.680	.049	-.407	-.267	5
Days 32° or below	5					1	-.498	.423	.283	4
Total Precipitation	6						1	-.118	.055	6
Testes Scrotal Non-Scrotal	7							1	.287	3
Adrenal Weight	8								1	

N - 120, d f. 118 Critical Valve at 5% Level - .180

16.6% of the variation in adrenal weights was accounted for by using all 7 variables and multiple regression techniques.

*Column identification is the same as for rows.

DISCUSSION

The fact that formalin fixation maintained the color of the adrenals as observed in freshly dissected specimens suggests that cortical lipoid was maintained at normal density, hence histological examination estimated adrenal lipoid at actual physiological concentrations.

Variability in the proportion of adrenal sections occupied by cortical tissue and the relatively consistent amount of medullary tissue suggests that differences in adrenal weights reflect changes in the amount of cortical tissue. This corresponds to the findings of Selye (1936), Christian (1950) and others. It is also in accord with the theoretical concept of changes in amount of adrenocortical tissue in response to stress (Christian 1955), i.e., stress is reflected in increased amounts of cortical tissue.

Cortical cells present in the medulla comprised a small portion of the total cortical substance, consequently it is believed their functional capacity is neglegible.

Interpretation of histological information in terms of cortical lipoid mobilization and subsequent accumulation in response to continuous stress as first outlined by Selye (1957), provided systematic criteria for evaluating the significance of the observed variations in cortical lipoid.

The endocrine mechanisms of reproduction in both the male and female mammal are very complex. Socio-psychological and physiological responses elicited by initiation of the breeding season have been shown to be interpreted by the organism in terms of physical stress (Chitty 1954, Christian 1957); which is ultimately reflected in characteristic adrenal changes. Pregnancy, according to Christian (1950), is capable of eliciting the adaptation reaction.

The reproductive season of the black-tailed jack rabbit in western Kansas begins in early winter. Hence at a time when environmental stressors are maximal there suddenly is an additional stimulus taxing the adaptation mechanism of the organism—directly by competing for pituitary function and indirectly by constituting a non-specific systemic stressor.

The presence of less lipoid, by zones, in adult pregnant females as compared with non-pregnant adult females suggests that pregnancy in the black-tailed jack rabbit is a stress factor. Assuming that pregnancy initiates the adaptation reaction, the high frequency of uniform staining in pregnant adult females at less than half term is accounted for in terms of Selye's observation (1937) that lipoid granules reappear at the end of the alarm stage and are evenly distributed throughout the various layers of the cortex. As adaptation continues, according to Selye (1937), the lipoid materials localize more particularly in the zona fasiculata, which in rabbits corresponds to zone B. This theory may account for the relatively high frequency of absence of staining in the outer zone of the cortex in pregnant adult females at half term or over-which represent a later stage of adaptation. Failure of non-pregnant adult females to demonstrate lack of staining in zone A, lends significant support to the proposed relationship of pregnancy to adaptation.

The rather broad categorizing of stages of pregnancy may account for the considerable degree of lipoid variation within the groups presented. It seems probable that should the stages of pregnancy be followed more closely the various phases of adaptation in terms of lipoid density and distribution could be more critically defined.

A small proportion of scrotal adult males exhibited very low total

lipoid. Non-scrotal adults showed a minimal total lipoid density somewhat higher. This suggests, according to the theory of adaptation, that activation of the testes constitutes a physiological stress. However, the mode total-lipoid-index-number for non-scrotal males was less than for scrotal males. The relatively high frequency of uniform staining in scrotal males as compared with non-scortal males again suggests activation of the adaptation mechanism. It was concluded that stimulation of the testes probably presented an additional stress to the hare but the evidence was not conclusive.

Compared with the adult groups, juveniles tend to show less lipoid within zones. This suggests that extrinsic stressors may particularly augment juvenile adrenal response. The relatively high frequency of juvenile males and females demonstrating uniform staining of the cortex also supports the thesis that juveniles respond more readily to environmental stressors than adult animals. The particular sensitivity of juveniles to stress factors may be important in terms of explaining population crashes since one of the basic requirements for such phenomena is the failure of young to survive to maturity. Adams (1959) has noted that trap sickness affected many more young than adults. This differential in total lipoid may be an age-class characteristic, however.

The slightly greater total cortical lipoid content of adult females than adult males and juvenile females than juvenile males was in accord with work by Whitehead (1934), and is probably a characteristic sex difference.

The sexual difference in adrenal size was in accord with work by Jackson (1913), who showed the adrenals of female albino rats to be larger than male adrenals. It is thought that this consistent adrenal weight differential is a sex characteristic and is independent of stress factors since the same

relationship was demonstrated in sexually immature specimens.

The concept of adrenal response to non-specific stressors as developed by Selye (1936), provided the basis for interpreting the variations in adrenal weight. Christian and Davis (1955) have postualted that augmented production of adreno-cortical hormones in response to stress is reflected in increased amounts of adreno-cortical tissue. Consequently the amount of cortical tissue, as measured by adrenal weight, is an indirect measure of stress.

The greater mean adrenal weight demonstrated by pregnant adult females compared with non-pregnant adult females, suggests that pregnancy was a definite stress factor within the black-tailed jack rabbit female population. Tarantine and Cassano (1955) working with rats have also shown that pregnancy results in increased adrenal weight.

Consideration of male and female reproductive responses in respect to adrenal weight variations, in this correlation study, must be prefaced by noting the relationship between age and adrenal weight since age groups were pooled for statistical analysis; and only adult individuals are capable of reproduction. The mean adrenal weight of adult females was significantly higher than for juvenile females. Consequently positive correlation between reproductive factors and adrenal weight is expected. Assuming that pregnancy constitutes a non-specific stressor--as evidenced by histological information and the differential in adrenal weights of pregnant and non-pregnant adult females--the significance of reproductive factor correlations with adrenal weight may be best interpreted in terms of relative values or rank of the variables in accounting for adrenal weight variation. Also,

since considerable adrenal weight variation in both sexes was not accounted for, these values must be considered within the limits of the explained variation.

Females that had produced one or more litters demonstrated the greatest tendency toward increased adrenal weight. This supported the proposed thesis of adaptation and pregnancy, suggesting that nulliparous individuals are not exposed to the stress of pregnancy, and hence do not show this increased adrenal weight. Positive correlation of body weight and adrenal weight demonstrated that adrenocortical tissue occurs in direct proportion to the size of the organism. Body weight in turn showed positive correlation with the number and stage of gestation of the embryos. This suggested that the advent of pregnancy and concomittant increased body weight may result in an increased adrenal weight indopendent of stress factors.

Negative correlation of average temperature and adrenal weight and positive correlation of frost days and adrenal weight, suggested that cold weather was a definite stress factor in the black-tailed jack rabbit population. Failure of precipitation to correlate significantly with adrenal weight suggested that under seasonally normal conditions black-tailed jack rabbits do not respond to periods of relative wetness or dryness. Activation of the testes constituted a physiological stress as evidenced by the high positive correaltion with adrenal weight. Body weight and age were also important factors determing male adrenal weight, however, it was concluded that adrenal weight variation within groups was largely the result of stress factors.

Failure to account for more of the variation in adrenal weight obviously suggests that there were other influencing factors in the population which

were not considered. It is thought that a more critical analysis of environmental and biotic stress factors could explain considerably more adrenal weight variation.

SUMMARY

(1) Macroscopically, adrenals usually appeared white or light yellow suggesting the presence of fatty substances.

(2) Sudanophilic material of the adrenal gland was restricted to the cortical tissue, however, cortical cells were frequently found mixed with medullary tissue.

(3) The cortex demonstrated three distinct zones according to Sudan III staining intensity. The outer area characteristically had less lipoid.

(4) Variations in adreno-cortical lipoid density and distribution with stages of reproductive activity of both males and females suggested activation of the adaptation mechanism.

(5) Juveniles demonstrated a slightly lower total cortical lipoid content than adults.

(6) Adrenal glands of female jack rabbits contain more stainable lipoid than males.

(7) Adrenal weight was largely determined by the amount of cortical tissue.

(8) Pregnant adult females demonstrated a greater mean adrenal weight than non-pregnant adults suggesting that pregnancy was a stress factor.

(9) Female adrenals were larger than male adrenals within both juvenile and adult age groups.

(10) Multiple regression techniques account for 18.6 per cent of the varia-

tion in male adrenals and 52.4 per cent in females.

(11) Simple correlation of biotic and environmental variables with adrenal weight showed temperature, pregnancy, age and body weight to be particularly important factors in determing adrenal weight.

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APPENDIX

EXPLANATION OF PLATE I

Black-tailed jack rabbit adrenals 3 X magnification.

Upper: Pair of adrenals taken from an adult male (left) and an adult female (right).

Lower: Each is from a different juvenile jack rabbit. Variation in adrenal size within an age group, and color differences are demonstrated.

PLATE I

EXPLANATION OF PLATE II

Fig. 1. Photomicrograph of adrenal gland taken from a pregnant adult showing enlarged cortex and cortical cells in the medulla.

Fig. 2. Photomicrograph of adrenal gland taken from a male juvenile showing uniform staining throughout the cortex and relative lack of cortical tissue.

PLATE II

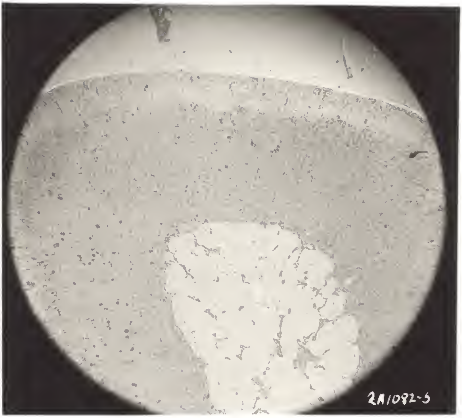

Fig. 1.

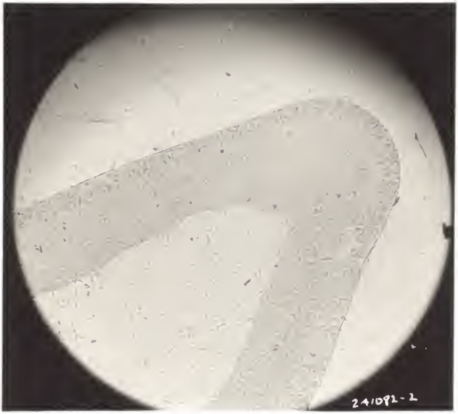

Fig. 2.

EXPLANATION OF PLATE III

Fig. 1. Photomicrograph of adrenal gland taken from a female juvenile demonstrating an absence of staining in zone A and a cortical anomaly.

Fig. 2. Photomicrograph of adrenal gland taken from a pregnant adult showing cortical invagenation.

PLATE III

Fig. 1.

Fig. 2.

ADRENAL RESPONSES IN A BLACK-TAILED JACK RABBIT
(*Lepus californicus melanotis*) POPULATION

by

NEIS C. ANDERSON JR.

B. A., Concordia College, 1958

AN ABSTRACT OF A THESIS

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1960

Periodic eruptions of Black-tailed Jack Rabbit (Lepus californicus melantois) populations are significant economically in terms of crop damage and also present an interesting ecological phenonomenon. This project was designed to study several phases of the ecology of a particular black-tailed jack rabbit population in western Kansas. Studies were conducted on reproduction, pathological conditions, parasitism and physiological responses in terms of adrenal glands. The latter area the basis for this investigation.

Physiological responses in wild populations to extrinsic and intrinsic factors are currently of particular interest to field biologists attempting to attribute cyclic mortality to a functional disorder. Indirect study of physiological responses through adreno-cortical enlargement, in terms of increased adrenal weight, and discharge of cortical lipoids which are well confirmed evidences of stress, greatly facilitates such investigations. This study was designed to elucidate adrenal response of black-tailed jack rabbits to such biotic and environmental characteristics as could be identified and accurately measured within the population. As study was made of histologically demonstrable adrenal lipoids and adrenal weights with respect to age groups, sex, reproduction and a variety of extrinsic factors as temperature and precipitation.

Macroscopically, the adrenals usually appeared white or light yellow in color suggesting the presences of lipoid materials. Histological examination showed great variability in the proportion of the adrenal occupied by cortical tissue as compared with the relatively constent amount of medullary tissue. From this it was concluded that adrenal weight is largely dependent upon the amount of cortical tissue in the gland.

Sudan III staining revealed that sudanophilic materials were restricted to the cortical tissue. Cortical cells were also frequently observed mixed with medullary tissue, however, they formed a relatively small proportion of the total cortical volume.

Staining showed the cortex to be divisible into three zones according to staining intensity. These areas were disignated A, B, and C. Lipoid density within zones was determined microscopically be assigning a numerical value of 0-3 to the individual areas according to the staining intensity.

Variations in adreno-cortical lipoid density and distribution with stages of reproductive activity of both males and females suggested activation of the adaptation mechanism. It is thought, therefore, that normal physiological reproductive activity, pregnancy in particular, constitute stressful stimuli to black-tailed jack rabbits. It was also demonstrated that female jack rabbits have more adrenocortical lipoids than males.

Adrenal weights showed definite variations with age and sex.

Adult Female Pregnant	Adult Female Non-Pregnant	Adult Male	Juvenile Female	Juvenile Male
388.4	378.8	270.0	243.9	229.3
383.6			236.2	

Figures are mean total weights of left and right adrenals in milligrams.

Multiple regression techniques accounted for 16.6 per cent of the variation in male adrenal weights using 9 variables. Of these factors frost days (32°F or below), body weight and age correlated most significnatly with adrenal weight. Using 7 variables 52.4 per cent of the varistion in

female adrenal weights was accounted for. Age, pregnancy and body weight ranked highest among simple correlations of these variables with adrenal weight. It was concluded that temperature, pregnancy, age and body weight are particularly important factors in determing adrenal weight.

SUMMARY

(1) Variations in adreno-cortical lipoid density and distribution with stages of reproductive activity of both males and females suggested activation of the adaptation mechanisms.

(2) Adrenal glands of female jack rabbits contain more stainable lipoid than males.

(3) Adrenal weight was largely determined by the amount of cortical tissue.

(4) Pregnant adult females demonstrated a greater mean adrenal weight than non-pregnant adults suggesting that pregnancy was a stress factor.

(5) Female adrenals were larger than male adrenals within both juvenile and adult age groups.

(6) Multiple regression techniques account for 16.6 per cent of the variation in male adrenals and 52.4 per cent in females.

(7) Simple correlation of biotic and environmental variables with adrenal weight showed temperature, pregnancy, age and body weight to be particularly important factors in determining adrenal weight.